EFFECT OF DIFFERENT ALBUMIN-BOUND FATTY ACIDS ON FATTY ACID AND CHOLESTEROL BIOSYNTHESIS IN RAT HEPATOCYTES

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1. Introduction and summary

Isolated rat hepatocytes have been used for the study of hormonal [1] and other [2,3] effects on hepatic fatty acid- and cholesterol biosynthesis, and extensive studies in the same field have been performed with suspended hepatocytes from neonatal chicks [4–6]. In a study from this laboratory [3] the rate of cholesterol biosynthesis from ^3H_2O in rat hepatocytes was found to compare well with perfusion data from other laboratories, whereas the rate of fatty acid synthesis was about ten times lower than in the intact organ. However it was possible to demonstrate an efficient feed-back inhibition of the fatty acid biosynthesis by adding albumin-bound oleic or palmitic acid. In the present study the inhibitory effect on fatty acid synthesis of albumin-bound fatty acids, varying in chain length from 8 to 18 carbon atoms and in degree of unsaturation was compared under incubation conditions that gave higher basic rates of lipogenesis. The use of a physiological buffer containing 25 mM bicarbonate instead of phosphate or HEPES (*N*-2-hydroxyethyl-piperazin-*N'*-2-ethanesulfonic acid) as major buffering substance increased the incorporation of ^3H_2O into fatty acids 3–4-fold, with little change in the rate of cholesterol synthesis. By addition of pyruvate or lactate the rate was further increased 2–3 times. Under these incubation conditions the ability of different albumin-bound fatty acids to inhibit fatty acid synthesis varied both with chain length and degree of unsaturation. At equivalent concentrations stearic acid was a more efficient inhibitor than unsaturated 18 carbon fatty acids and shorter saturated fatty acids. Since stearic acid is utilized by the cells at a lower or similar rate compared with the other fatty acids [6,7], the data suggest that the inhibitory action is exerted at an early stage of its metabolism and is not a consequence of its esterification or oxidation. In 0.5 mM concentration the fatty acids had no significant effect on the cholesterol biosynthesis from ^3H_2O in short term incubations.

2. Methods

Hepatocytes were isolated from fed rats on a controlled feeding schedule [3], essentially according to the method of Berry and Friend [8]. The same experimental setup was used as in a previous study [3], but hyaluronidase was omitted, 1% defatted [9] and dialyzed bovine serum albumin was added, and the collagenase concentration was lowered to 0.02% [10]. After 15 min perfusion 1.25 mM $CaCl_2$ was added to the Ca^{2+}-free medium and the perfusion was continued for another 15 min. The cell viability was estimated by trypan blue exclusion and varied between 80% and 98%.

Incubations were performed in 25 ml Erlenmeyer flasks in a total vol of 1.5 ml or in tissue culture flasks in a total vol of 2.5 ml, under the conditions described in the result section. When buffers containing 25 mM HCO_3^- were used, incubations were performed under an atmosphere of 5% CO_2, 95% O_2. Other incubations were performed under air.

The reactions were interrupted, and the incorporation of ^3H_2O into fatty acids and cholesterol was determined as described earlier [3]. Protein was determined according to Lowry et al. [11]. Pyruvate

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and lactate were determined by enzymatic fluorimetric methods [12].

3. Results and discussion

In two initial experiments the rate of fatty acid biosynthesis in suspended rat hepatocytes was 3.3 and 3.6 times higher in physiological buffers containing 25 mM $NaHCO_3$ than in bicarbonate free media buffered with 10 mM phosphate or 19.4 mM HEPES (table 1). 4.2 mM $NaHCO_3$ which is commonly used in buffers designed for incubation under air, stimulated the fatty acid synthesis only 1.2–1.6 times when added to the HEPES- or phosphate-buffered media. On the basis of these data a buffer containing 25 mM $NaHCO_3$, and 19.4 mM HEPES to increase the buffer capacity, was used in the subsequent experiments. The concentration of NaCl was lowered to 5.6 g per liter to retain isoosmolarity. In a total of ten experiments with this buffer the incorporation of ^3H_2O into fatty acids was 50.4 ± 6.5 (S.E.M.) ng-atoms/mg protein/hr as compared to

Table 1
Fatty acid biosynthesis in media with different bicarbonate concentrations

Buffer	ngatoms ^3H/mg protein in fatty acids	
Experiment no.	1	2
Krebs–Ringer bicarbonate	34.2	43.8
Bicarbonate free Hanks' solution containing:		
a) 10 mM phosphate	13.5	12.5
b) 19.4 mM HEPES	14.1	13.3
Hanks' solution containing:		
a) 10 mM phosphate + 4.2 mM HCO_3^-	20.9	16.8
b) 19,4 mM HEPES + 4.2 mM HCO_3^-	16.1	16.0
c) 19,4 mM HEPES + 25 mM HCO_3^-	49.8	42.4

In two experiments 11.7 and 8.6 mg cell protein was incubated for 60 minutes in a total volume of 2.5 ml buffer containing 1% defatted and dialyzed bovine serum albumin. 1.5 mC^3H_2O was added to each incubation. The values are means of duplicate incubations.

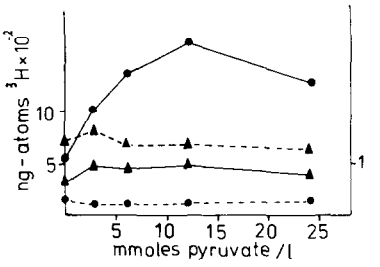

Fig. 1. Effect of pyruvate on fatty acid and cholesterol biosynthesis. In the first experiment (——) 12.1 mg cell protein was incubated for 60' with 1.5 mCi ^3H_2O in 2.5 ml Hanks' buffer containing 19.4 mM HEPES and 25 mM $NaHCO_3^-$. In the other experiment (- - - -) 19.0 mg cell protein was incubated under the same conditions except that bicarbonate- and HEPES-free Hanks' buffer was used. The medium was buffered with 10 mM phosphate in this experiment. Circles = fatty acids (left scale). Triangles = cholesterol (right scale).

17.5 ± 4.0 ng-atoms/mg protein/hr in an earlier study where Hanks' solution buffered with phosphate was used [3]. The incorporation into cholesterol was 11.6 ± 2.7 ng-atoms/mg protein/hr as compared to 11.6 ± 2.1 in the earlier study. The reason for the stimulatory effect of bicarbonate is not clear from the present data, but may depend on the requirement of HCO_3^- for the acetyl-CoA carboxylase reaction. At least the data presented in fig. 1, showing that pyruvate-stimulated fatty acid synthesis significantly in bicarbonate, but not in bicarbonate-free buffers, indicate a direct effect of bicarbonate at a step beyond the production of pyruvate and lactate.

Even in the bicarbonate rich medium the cells differ from the perfused rat liver in the sense that the fatty acid – as well as the lactate – and pyruvate production were increased to a lesser extent by increasing the glucose concentration in the medium (fig. 2). For instance in the perfused rat liver Brunengraber et al. [13] found a 2.5-fold stimulation of fatty acid synthesis and about a 2-fold increase in pyruvate and lactate concentration of the perfusion medium, when 15 mM glucose was added, and no further stimulation at higher glucose concentrations. In our experiments even very high glucose concentrations produced less than 50% increase in fatty acid synthesis and only a small increase in lactate and pyruvate production (fig. 2). The reason for this difference is not clear. However, the rate of glycolysis rather than loss of the

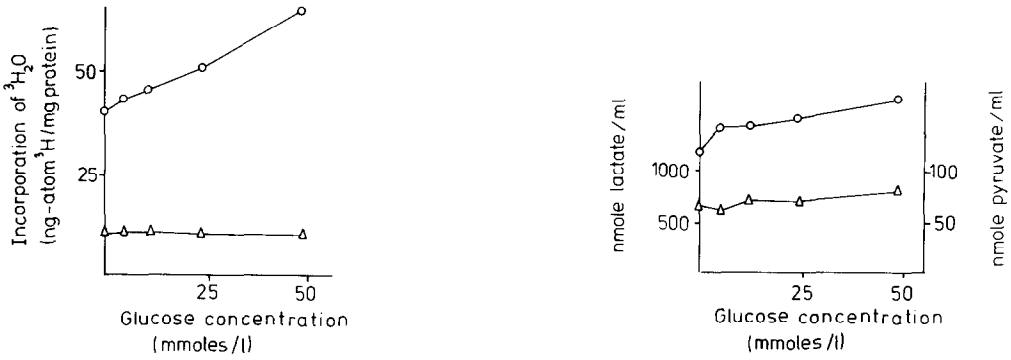

Fig. 2. Effect of increasing glucose concentration on fatty acid and cholesterol biosynthesis. 15.0 mg protein was incubated for 60′ in Hanks' buffer containing 19.4 mM HEPES, 25 mM $NaHCO_3$ and 1% bovine serum albumin. Left figure: (o—o) fatty acids; (Δ—Δ) cholesterol. Right figure: (o—o) lactate; (Δ—Δ) pyruvate.

ability to synthesize fatty acids may be the reason why the basic rate of fatty acid synthesis was lower than in the perfused liver, since the incorporation of ^3H_2O into fatty acids was increased 2.5–3-fold by addition of pyruvate or lactate (fig. 1). This effect was apparent already at 5 mM concentration, i.e. the concentration seen in the perfusion experiments by Brunengraber et al. [14] at 15 mM glucose concentration.

At optimal incubation conditions i.e. in a medium containing both 10 mM pyruvate and 25 mM bicarbonate the effect of equimolar concentrations of different albumin-bound fatty acids was tested. This study showed that the degree of feed-back inhibition of the fatty acid synthesis depended both on the chain length and the degree of unsaturation. Stearic acid was the most efficient inhibitor despite the fact that this fatty acid is utilized at a lower rate than other fatty acids in hepatocyte suspensions [6,7], and at the same rate in the perfused rat liver [14]. The low rate of utilization of stearic acid in combination with its strong inhibitory effect supports earlier suggestions [3,6] that the inhibitory effect must be exerted already when the fatty acid is present as free fatty acid or as acyl-CoA derivative in the cell, but leaves no information about the metabolic steps at which the action occurs. However, the degree of inhibition caused by the different fatty acids (>18:0 >18:1 >16:0 >14:0 >10:0) followed the same order as the strength of the inhibitory effect caused by different acyl-CoA derivatives on the acetyl-CoA

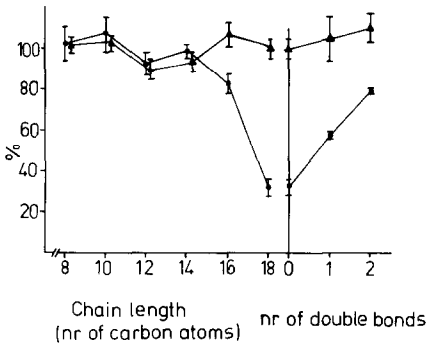

Fig. 3. Effect of different albumin-bound fatty acids on the incorporation of ^3H_2O into fatty acids and cholesterol. 13.4–20.0 mg cell protein was incubated with 1.5 mCi ^3H_2O for 60 min in the presence of 0.5 mM fatty acids bound to 1% bovine serum albumin. The incubation medium consisted of 1.5 ml of Hanks' solution buffered with 25 mM bicarbonate and 19.4 mM HEPES, containing 10 mM Na-pyruvate. The figures show the incorporation of ^3H into fatty acids (●—●) and cholesterol (▲—▲) expressed as % of the incorporation without fatty acid addition. In the left figure is shown the effect of different saturated fatty acids with increasing chain length. In the right figure the effect of stearic, oleic and linoleic acid are compared. All data are means ± S.E.M. of three experiments.

carboxylase reaction in vitro reported by Numa et al. [15], which adds circumstantial evidence for a physiological inhibitory role of long-chain acyl Co-A's at the acetyl-CoA carboxylase step. The finding that the cholesterol biosynthesis was not significantly

affected by the addition of 0.5 mM fatty acids during short term incubation is in line with the earlier studies on suspended hepatocytes [3] and perfused rat livers [16].

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